The Influence of Skin Structure on Permeability: An Intersite and Interspecies Comparison with Hydrophilic Penetrants

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For reasons that are unclear, skin from various body sites has different permeability properties. We have used hydrophilic penetrants (water, ethanol, mannitol, and paraquat) to study the in vitro permeability of skin from marmoset (eight body sites), man, and rat. Skin structure (stratum corneum thickness and number of cell layers; epidermal and dermal thickness; number and area of hair follicle openings per mm^2) was compared with permeability. There was no apparent relationship between skin structure and permeability to the most rapid penetrants, water and ethanol. Follicle area opening was the structural feature that varied most between species and between body sites. Different marmoset body sites showed a threefold range in follicle area but this did not appear to influence the absorption rates of the test penetrants. However, among the species there was an 80-times range in follicle area, which correlated with the observed differences in rate of mannitol and paraquat absorption. Thus, permeability could be related to inter-species differences in skin structure, but only with the relatively slowly absorbed test penetrants, mannitol and paraquat. *J Invest Dermatol 96:921–925, 1991*

Drugs can be deliberately applied to skin at different sites of the human body, either to treat abnormal skin [1] or to effect absorption into the systemic circulation [2]. Industrial chemicals and agrochemicals may come into contact with skin whether it is unprotected [3] or covered by clothing [4]. Protocols to assess the toxicity or efficacy of compounds, or to assess percutaneous absorption, invariably stipulate application to a specific body site. However, it has been reported that for a number of compounds the rate and extent of absorption through skin can be influenced by the site of application [5]. Indeed, this variability in site permeability has been deliberately and effectively exploited, for example, in the post-auricular placement of transdermal drug delivery devices [2]. In contrast, the absorption of some chemicals is not influenced by body sites. For example, the herbicide paraquat is absorbed at a similar low rate following application to hand, leg, and forearm [6].

The magnitude of general body site permeability differences and any subsequent effect on systemic delivery has not been studied extensively. Quantitation of these differences, for a range of chemicals, would allow a better understanding of the likely systemic uptake and subsequent hazards from dermal exposure. Therefore, we investigated the differences in the permeability of eight major body regions (scalp, upper arm, forearm, back, thorax, abdomen, thigh, and lower leg) of the marmoset, *Callithrix jacchus*. Four water-soluble test penetrants were used: tritiated water (subsequently referred to as water), ethanol, mannitol, and paraquat dichloride (referred to as paraquat). We have shown previously [7] that the skin of the more commonly used laboratory animals can be poor models for human skin permeability, whereas the use of primate skin has been advocated for obtaining data more relevant to man [8,9]. Thus, data derived with the marmoset in this study should provide a better indication of the magnitude of body site permeability differences to be found in humans.

Absorption was measured in vitro through excised skin; this technique has been used previously to predict in vivo absorption [9–11]. The skin structure of each body region was examined histologically to quantify the number and thickness of stratum corneum cell layers, the thickness of the epidermis and the dermis, and the number and size of hair follicles. These data have been used to explore the relationship between skin structure and permeability. In addition, the histology and absorption characteristics of human and rat skin were studied to identify any inter-species relationships between permeability properties and skin structure.

MATERIALS AND METHODS

Test Penetrants All radiochemicals were supplied by Amersham International PLC, Amersham, Bucks, UK. [Methyl-^{14}C] paraquat dichloride, D-[1-^{14}C] mannitol, and [1-^{14}C] ethanol were added to 1 mg ml^{-1} aqueous dilutions to final specific activities of 6.26, 4.47, and 2.7 μCi ml^{-1} respectively. [^{3}H] water was added to physiologic saline to a final specific activity of 2.8 μCi ml^{-1}.

Skin Preparation Human abdominal skin was obtained from cadavers within 24 h of death. Subcutaneous fat was removed and the skin used immediately or stored in aluminium foil (at 4°C for up to 7 d) before use. In this study, up to five pieces of skin, for mounting in a diffusion chamber, were obtained from each sample of human skin used (20 samples in total). Rats (Wistar derived, Alpk/AP strain, 80–100 g) and marmosets were supplied by the Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Nr Macclesfield, UK. Rats were killed by inhalation of Fluothane (Halothane BP, ICI Pharmaceuticals, Wilmington, DE) followed by cervical dislocation. The marmosets were killed by intraperitoneal injection of Euthatal, (Pentobarbitone sodium BP, Rhone-Poulenc Ltd, Dagenham, UK). The skin from the marmosets was removed from eight different body areas (abdomen, back, forearm, lower leg,

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thigh, scalp, thorax, and upper arm). Skin from the rat was removed from the dorsal surface. Subcutaneous fat and any muscle was removed from the rat and marmoset skins and they were then treated in the same manner as the human skin.

Percutaneous Absorption Assessments Skin samples were mounted in glass diffusion chambers that consisted of an open-top donor chamber and receptor chamber (volume approximately 5 ml) [12]. These chambers exposed a 2.54 cm^2 area of skin. The initial step was to test the integrity of each sample that might have been damaged during handling or storage. The receptor chambers were filled with saline and then tritiated water ($>100\ \mu l\ cm^{-2}$) was added to the donor chambers. The diffusion chambers were placed in a water bath maintained at 30°C (±1°C) throughout all the experiments. Samples were taken over a 6-h period from the receptor chamber (an equal volume of fresh receptor fluid was added to maintain the volume) and analyzed for radioactivity (BETAmatic liquid scintillation counter, Kontron Instruments Ltd, Welwyn Garden, UK). The receptor fluid was mixed thoroughly by a small magnetic stirring bar. At the end of the 6-h period the donor and receptor chambers were emptied and washed with saline. A small volume of saline (<0.5 ml) was placed in the receptor chamber and the cells left overnight. In this manner the skin membranes were allowed to hydrate naturally, i.e., stratum corneum surface exposed to the atmosphere and dermal surface to a high relative humidity.

From the results of the assays, graphs were drawn of the amount of tritiated water penetrated versus time. From the linear portion of such a graph, a steady-state absorption rate (J_s) was calculated. The permeability for each membrane was expressed by the calculation of a permeability coefficient (K_p, units cm h^{-1}) thus,

$$K_p(\text{cm h}^{-1}) = \frac{J_s\ (\text{amount absorbed cm}^{-2}\ \text{h}^{-1})}{\text{applied concentration (amount cm}^{-3})}.$$

The following day this procedure was repeated with the test penetrant solutions applied to the skin samples in the donor chamber. Again, the final expression of the permeability to each of the test penetrants was a calculated permeability coefficient.

Determination of Stratum Corneum and Epidermis Thickness in Man, the Marmoset, and the Rat Samples of human abdominal cadaver skin, (men age 22, 48, 51, 54, and 71 years; women age 13, 47, 68, 75, and 75 years) were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). Similarly, sections of skin were removed from the forearm, upper arm, thorax, abdomen, thigh, lower leg, back, and scalp of five marmosets and from the dorsal region of eight male, 28-day-old rats.

After 24 h fixation, the samples were post-fixed in 1% osmium tetroxide, dehydrated in graded acetone and embedded in Araldite. A 1 μm transverse section, stained with 1% toluidine blue (in 1% borax) was examined by light microscopy. Quantitative measurements of the thickness of the stratum corneum and epidermis were achieved using a Leitz Imagan 2 image analysis system (Leica U. K., Milton Keynes, England).

Determination of the Number of Hair Follicles Per Unit Surface Area

Human and Marmoset Skin: Shaved samples of human abdominal skin and marmoset skin (forearm, upper arm, thorax, abdomen, thigh, lower leg, back, and scalp) were photographed using the bellows attachment on a Nikon FG 20 camera (magnification ×30). Values were obtained for the number of follicles by counting the number of hairs within a known calibrated area.

28-Day-Old Rat Skin: Whereas human and marmoset hairs were readily identifiable in photographs of the skin, those of 28-day-old albino rats were more difficult to distinguish. In order to overcome the inherent lack of contrast in albino hairs, samples of shaved rat skin (five per animal) were mounted on glass slides and photographed using a fluorescence light microscope (Polyvar excitation filter, 455–490 nm). Values were obtained for the number of follicles by counting the number of hairs within a known calibrated area.

RESULTS

Permeability Determinations The absorption rate data, expressed as permeability coefficient values ($K_p \times 10^{-4}$ cm h^{-1}) are summarized in Table I. Each presented value has been derived by determining the mean value of several determinations from each piece of tissue from a single animal and then calculating the final mean value for all the animals in the group. The maximum range in absorption rates for a single penetrant through the body areas was detected with paraquat (7.7-times range). Water and ethanol were the most rapid penetrants. Both mannitol and paraquat were absorbed at very similar rates, but more slowly than the other two penetrants. There was no apparent pattern to the body site permeability, i.e., the body areas could not be "ranked" according to the absorption of the four penetrants studied. Water and ethanol were absorbed at similar rates through rat skin. In contrast to marmoset skin, the fastest rat skin penetrant was mannitol and the slowest was paraquat. The penetrants were absorbed through human and marmoset skin at rates in the decreasing order of water > ethanol > mannitol > paraquat.

The inter-species skin permeability values were all similar for water and ethanol. The mannitol absorption rate was also similar in skin from both the marmoset and human body areas. In contrast, the mannitol absorption rate through rat skin was seven times or more greater than through the other skin preparations. The greatest range of permeability values was detected for paraquat with rat skin more than 30 times more permeable than human abdominal skin, and twice as permeable as the most permeable body area (forearm) of the marmoset.

Histological Examinations/Structural Parameters Details of the data collected for the various histologic parameters studied are collated in Table II. The measured thickness of epidermis for each of the marmoset body sites showed a very narrow range (16.94 to 21.61 μm). The human epidermis (Fig 1*a*) was thicker than the rat (Fig 1*b*) and the marmoset body sites. A twofold range was found for the dermis between the thinnest marmoset body site (lower leg)

Table I. The Permeability[a] of Different Body Areas to Hydrophilic Penetrants

Species	Body Area	Water	Ethanol	Mannitol	Paraquat
Marmoset	Abdomen	4.85 (2.54; 25)	3.78 (3.61; 3)	1.22 (0.60; 5)	0.85 (0.76; 15)
	Back	8.38 (3.35; 27)	3.18 (0.52; 3)	0.83 (0.32; 6)	0.64 (0.42; 17)
	Forearm	11.0 (4.32; 17)	8.04 (3.80; 6)	2.11 (1.95; 5)	1.61 (0.15; 2)
	Lower leg	6.72 (3.09; 19)	5.58 (1.95; 6)	0.89 (0.89; 6)	1.49 (0.62; 5)
	Scalp	9.75 (4.72; 13)	3.93 (1.13; 4)	0.49 (0.31; 4)	0.21 (0.10; 2)
	Thigh	7.65 (2.76; 16)	4.13 (1.76; 3)	1.43 (1.80; 6)	0.77 (0.24; 3)
	Thorax	6.67 (2.99; 24)	5.76 (2.58; 3)	2.00 (2.21; 5)	1.38 (1.15; 11)
	Upper arm	9.91 (4.55; 17)	6.54 (1.89; 5)	0.66 (0.89; 4)	1.16 (0.77; 4)
Rat	Back	4.85 (5.03; 12)	4.15 (1.88; 8)	13.4 (2.63; 4)	3.46 (1.42; 4)
Human	Abdomen	6.39 (3.24; 24)	3.17 (1.20; 5)	0.61 (0.11; 3)	0.087 (0.08; 19)

[a] All values are mean permeability coefficients (Kp, units × 10^{-4} cm h^{-1}; figures in parenthesis are ± SD, number of skin samples).

Table II. Data of the Structural Parameters Examined for Each of the Skin Types Used in this Study[a]

Species	Body Area	Stratum Corneum Width (μm)	Number of Cell Layers	Mean Cell Layer Width (μm)	Epidermal Width (μm)	Dermal Width (μm)	Follicle Number (per mm²)	Follicle Diameter (μm)	Follicle Area (μm²/mm²)
Marmoset	Abdomen	10.29 (4.20; 5)	4.44 (0.59; 5)	2.32	17.73 (3.63; 5)	741.6 (308.1; 5)	7.37 (2.36; 3)	17.01 (2.60; 5)	1674
	Back	10.55 (3.33; 5)	4.87 (0.59; 5)	2.17	18.51 (4.60; 5)	741.7 (299.9; 5)	21.7 (—; 2)	17.27 (2.29; 5)	5083
	Forearm	10.54 (4.06; 5)	4.46 (0.58; 5)	2.36	21.61 (4.18; 5)	530.6 (156.6; 5)	16.2 (6.9; 3)	15.02 (2.76; 5)	2870
	Lower leg	9.81 (3.74; 5)	4.14 (0.33; 5)	2.37	18.46 (4.38; 5)	478.0 (190.9; 5)	11.9 (2.7; 3)	14.13 (1.41; 5)	1866
	Scalp	10.62 (3.76; 5)	4.74 (0.47; 5)	2.24	18.46 (4.78; 5)	796.9 (282.6; 5)	18.2 (6.9; 3)	17.48 (1.88; 5)	4367
	Thigh	10.66 (3.72; 5)	4.23 (0.77; 5)	2.52	17.12 (3.66; 5)	490.7 (200.3; 5)	10.3 (3.7; 3)	16.14 (2.59; 5)	2107
	Thorax	9.36 (3.11; 5)	3.89 (0.41; 5)	2.41	17.53 (3.96; 5)	1358.7 (516.4; 5)	11.0 (2.1; 3)	15.55 (1.95; 5)	2094
	Upper arm	10.23 (4.16; 5)	4.70 (0.67; 5)	2.18	16.94 (4.74; 5)	480.0 (160.6; 5)	6.7 (2.8; 3)	17.48 (4.25; 5)	1607
Rat	Back	16.4 (4.17; 8)			18.6 (2.68; 8)	784.7 (335.0; 8)	79.3 (10.7; 8)	18.32 (1.82; 8)	20903
Human	Abdomen	19.2 (7.05; 10)			25.5 (4.31; 10)	>2000	0.063 (0.0419; 4)	70.2 (55.4; 9)	243

[a] Each value is the mean; figures in brackets are SD; number of determinations.

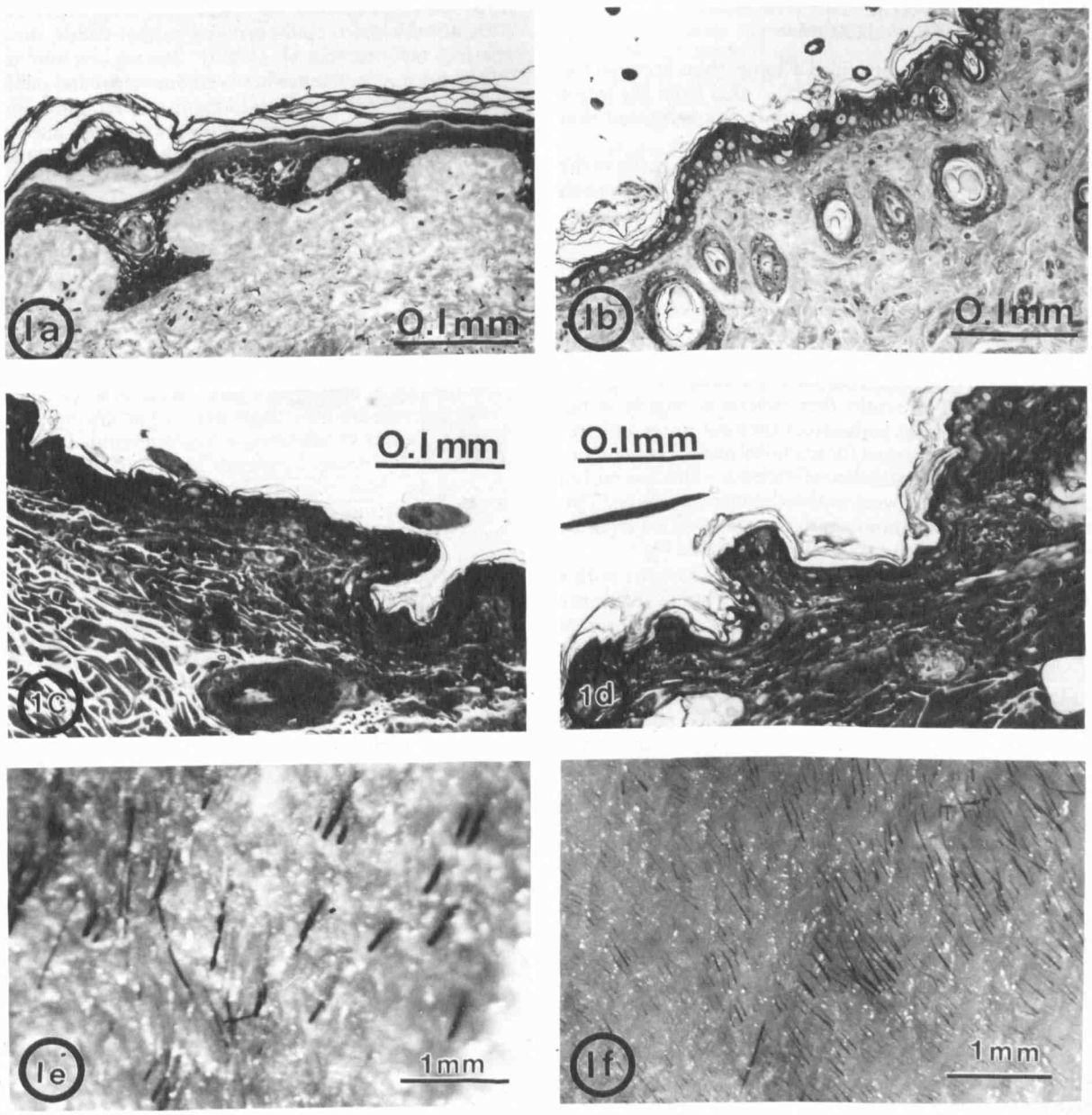

Figure 1. Photomicographs of some of the different skin types used in this study. *a* and *b*, human abdominal and rat back skin, respectively. *c–f*, examples showing the variations in some of the structural parameters in the marmoset body areas. *c*, thorax skin that had the thinnest stratum corneum; *d*, thigh skin that had the thickest stratum corneum. *e*, upper arm, which had the least hair follicles; *f*, back with the highest number of hair follicles.

and the thickest (thorax). The rat dermis had a similar thickness to the marmoset dermis in contrast to the human dermis, which was so thick (>2000 μm), that we did not quantify this accurately. The stratum corneum values showed the human skin to be the thickest, followed by rat and then the marmoset (Fig 1*c,d*). Even so, there was less than a factor of 2 range in the determined values. Thus, in the marmoset, the stratum corneum thickness, number of cell layers, and calculated thickness of each cell layer was almost constant over the body sites examined.

A wider range of values was seen among the species in the number of hair follicles and subsequent calculated areas of hair follicle opening on the skin surface. In the marmoset, there was an approximate threefold difference between the region with the least hair follicles/mm^2 (upper arm, 6.7, Fig 1*e*) and that with the highest number (back, 18.2, Fig 1*f*). These values were higher than found in the human abdomen (<1), but lower than seen in the dorsal skin of the rat (approximately 80). These differences resulted in a large species-related difference in the follicle area/mm^2 and there was an 80 times difference between the skin with the least follicle area (human) and that with the highest area (rat).

DISCUSSION

The data from this study have provided a comparison between the permeability of marmoset *(Callithrix jacchus)* skin from the major body sites and skin structure. An inter-species comparison of skin structure and permeability has also been made.

Differences in the permeability of each marmoset body site to the penetrants were measured (Table I). The results show that overall the absorption rate for water > ethanol > mannitol ~ paraquat, but there was no consistent pattern within the body regions. Each penetrant showed a range in the inter-site absorption rates with factors of difference between the least and the most permeable areas being between 2.3 (water) and 7.7 (paraquat). However, this range obtained for paraquat was greatly increased by the rate of absorption measured through the scalp, the least permeable area. Generally, the range measured with this penetrant through body sites was no greater than 2.5 times. The results demonstrate a ranking in the permeability of the marmoset body sites tested for water and ethanol, but no clear picture emerged for mannitol and paraquat. Considering all the data, the permeability of each body site was ranked highest in the forearm and lowest in the abdomen and back. This ranking differs from published human in vivo data, which reported the forearm to be least, and the scalp most permeable [8].

Factors of difference have been reported in other studies with a range of species and chemicals. A maximum fivefold difference detected through scalp skin compared with forearm, was reported from in vivo human studies with the penetrants hydrocortisone, malathion, and parathion using different body regions [13,14]. In another study, absorption through the scrotum was found to be 40 times more permeable to hydrocortisone and 12 times more permeable to the pesticides relative to the forearm [13,14]. In vitro studies examining the absorption of a homologous series of alcohols through hairless mouse skin showed less than a factor of 3 difference in the permeability of dorsal and abdominal skin with mice less than 50 d of age [15] and no differences with older mice. The dorsal and ventral skin of hairless mice had the same in vitro permeability to hydrocortisone regardless of age [16]. The range of values found for each penetrant and body site in our study seem, therefore, consistent with ranges reported in other studies.

Little variability was seen in the thickness of either epidermis or dermis from the marmoset, regardless of site (Table II). No correlation was found between the absorption rates, through marmoset skin, of any of the penetrants and the thickness of the epidermis and dermis. A significant correlation has been demonstrated for stratum corneum thickness and absorption of nitroglycerin [17] and water [18] through rat skin. In both cases absorption was greater through skin sites with a stratum corneum thickness only half that of the comparison site. No correlation was found between the thickness of the stratum corneum at each marmoset body site and the permeability of that site for each test penetrant (Fig 2*a*) in the present study. A similar result pattern was also found between the number of stratum corneum cell layers and permeability. The similarities in the permeability values may simply reflect the similar thicknesses of stratum corneum.

Before discussing further any relationships between permeability and skin structure, it is worth comparing the values obtained in this study, using the described methods of tissue preparation, with other reported values. The values determined in this study differ in some respects with other reported values. Using a frozen section technique, Bronaugh [19] found that human stratum corneum was 16.8 μm thick, which was in agreement with a previously reported value of 15.8 μm [20]. Somewhat lower values of 13 μm and 8.2 μm were found in human skin that had been dehydrated. Using our technique, we found similar values to those reported in the literature. Rat stratum corneum thickness in frozen sections has been reported to be 18.4 μm [19], which is slightly thicker than the values of 16.4 μm found in this study. However, our values for the thickness of human and rat epidermis (19.2 μm and 16.4 μm, respectively) are lower than previously reported [19] values (46.9 μm and 32.1 μm, respectively). When the values from this study, for the three species examined, are considered, it appears that for stratum corneum thickness, epidermal and dermal width, the human skin values > rat > marmoset.

The greatest difference between our values and published values is for the number of hair follicles in the rat and human per cm^2. However, there was closer agreement between our values for the diameter of rat and human follicles (18.32 μm and 70.2 μm, respectively) and previously published values (25 μm and 97 μm, respectively). It is possible that the differences in the number of follicles

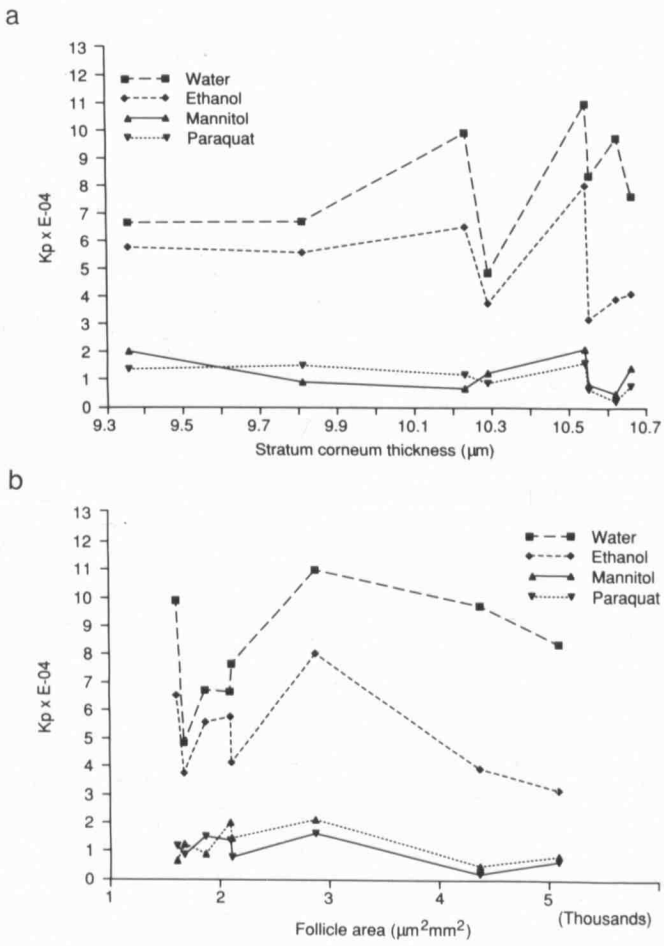

Figure 2. The relationship between permeability, stratum corneum thickness (*a*), and hair follicle opening area (*b*) for each of the eight marmoset body regions and the four test penetrants.

measured in the rat skin reflect strain and age differences in the rats examined in the various studies. Another explanation is that the method we have used to determine the number of follicles using a fluorescence light microscope is more sensitive, especially for use with albino animal skins.

In the marmoset, the widest range of structural parameter values was calculated for the follicular opening area per unit area of skin for each body site (Table II). The range of this parameter showed a factor of 3 difference between the site with the lowest opening area (upper arm) and the largest (back). Previous studies have indicated that hair follicles might influence absorption, e.g., epinephrine [21], mercuric and sodium chloride [22]. Although there was a threefold range in the follicular area among the marmoset body sites, there was no apparent effect on permeability (Fig 2*b*). This was not too unexpected for water and ethanol where diffusion through the whole of the stratum corneum is believed to be the route of absorption. However, with the more slowly absorbed mannitol and paraquat, follicular (or "shunt") diffusion was expected to be a more important component of the absorption pathway.

When inter-species comparisons were made between follicle opening area and permeability to the penetrants no relationships were detected with water and ethanol. A relationship, though, was seen with mannitol and paraquat (Fig 3). The human skin had the lowest follicular opening area, with the marmoset over 6 times and the rat over 80 times greater (Table II). The permeability values reflected this trend. With paraquat, for example, marmoset skin was 10 times and rat skin 40 times more permeable than human skin. These data suggest that in a particular species the permeability properties of skin are not controlled or measurably influenced by a single parameter. As parameters such as thickness of stratum corneum or number and area of hair follicles change, then permeability properties cannot or should not be assumed to change. Only when the values of the various parameters show much greater variation, as between species, might it be possible to relate skin structure to permeability.

Although it was not the intention of this study to explore fully the usefulness of the marmoset as a model for human skin permeability properties, a comparison has been made with the four test penetrants. There was no difference in the permeability values (Table I) for water, ethanol, and mannitol through human and marmoset abdominal skin. However, with paraquat, marmoset skin was 10 times more permeable than human skin. We previously reported [7] that the rat provides the closest model for human skin paraquat penetration even though permeability values for paraquat are still 40 times greater compared with human skin in vitro. These limited data, therefore, suggest that the marmoset may be a better model than many other laboratory animals for human skin permeability properties (at least for water-soluble molecules). This promotes confidence that the relationships detected between body site permeability, skin structure, and the magnitude of permeability differences of the marmoset body areas, might be relevant to man. The magnitude of these differences were compound dependent and slightly higher with the more poorly absorbed chemicals. However, in the marmoset, over the general body surface the major areas showed only small differences in permeability to water-soluble molecules.

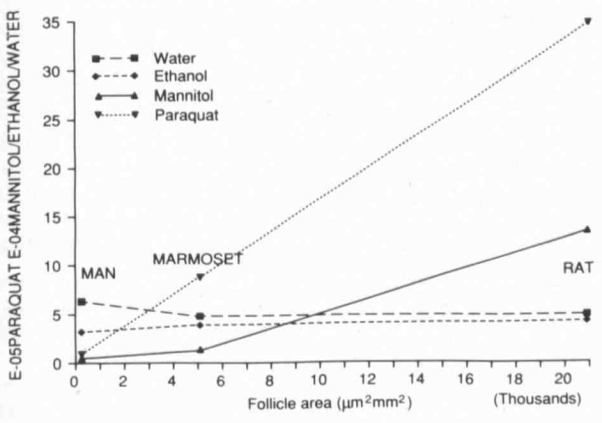

Figure 3. An inter-species (human, marmoset, rat) comparison between hair follicle opening area and permeability to the four test penetrants (permeability is expressed as permeability coefficient and units for water, ethanol, and mannitol are $\times 10^{-4}$ cm h^{-1} and for paraquat $\times 10^{-5}$ cm h^{-1}).

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